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ON THE OCCURRENCE AND DETECTION OF HÆMATOPORPHYRIN IN THE URINE.

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ON THE OCCURRENCE AND DETECTION OF HÆMATOPORPHYRIN IN THE URINE. By ARCHIBALD E. GARROD, M.A., M.D., F.R.C.P. (Plate XXIV.).

Of recent years a number of cases have been placed on record in which the urine was found to contain pigments exhibiting more or less obvious affinities with that derivative of hæmatin to which the name of Hæmatoporphyrin has been assigned.

Some of these urinary colouring matters were apparently identical with one or other of the varieties of Hæmatoporphyrin which have been prepared by different processes from blood pigment, whereas others have presented noteworthy differences from these, either in their spectroscopic characters or chemical properties.

The earliest observations bearing upon this subject date from the year 1874, when Baumstark[1] described two pigments which he obtained from the urine of a patient suffering from leprosy, and to which he gave the names of urorubrohæmatin and urofuscohæmatin respectively. Acid solutions of urorubrohæmatin yielded a spectrum bearing the closest resemblance to that of acid hæmatoporphyrin, but the alkaline spectra of the two substances presented a much less conspicuous likeness to each other. The pigment was insoluble in alcohol unless an acid were present; its acid alcoholic solution had a violet colour; and unlike hæmatoporphyrin it was not precipitated from alkaline solutions by salts of calcium or of barium. Baumstark, therefore, although he called attention to the resemblance of urorubrohæmatin to iron-free hæmatin, did not regard the two substances as identical.

In 1880 MacMunn[2] described a pigment obtained from the urine of a patient suffering from acute rheumatism, to which he at first assigned

[1] *Pflüger's Archiv*, 1874, ix. p. 568.

[2] *Proc. Royal Soc.*, 1880, xxxi. p. 211.

the name of urohæmatin. He found that the acid and alkaline solutions of this substance yielded spectra which were identical with those of one of the products of the action of nascent hydrogen upon hæmatin, the alkaline spectrum of which, although it bears a very obvious resemblance to that of hæmatoporphyrin, differs from it in showing five instead of four bands. The acid spectrum showed, in addition to the characteristic hæmatoporphyrin bands, a band situated between the *b* and *F* lines of the solar spectrum, very similar to that of urobilin.

In the following year (1881) Neusser[1] found a pigment in the urine of two patients, one of whom was suffering from Phthisis Pulmonalis, and the other from Pleurisy with effusion, which was spectroscopically identical with hæmatoporphyrin; and he came to the conclusion that it was probably the same substance. Both urines showed, when examined without any preparation, two bands very like those of oxyhæmoglobin, but the absence of proteid precluded the notion that any unchanged blood pigment was present. It is noteworthy that Baumstark's specimen yielded a similar spectrum, and the same bands have been seen by several more recent observers.

In 1883, MacMunn[2] recorded some further cases of acute rheumatism and a case of so-called idiopathic pericarditis, in which urohæmatin was present in the urine; and in a paper published in 1885[3] he changed the name of his pigment to the more appropriate one of urohæmatoporphyrin.

In 1887 Le Nobel[4] confirmed MacMunn's observations of the occurrence of a pigment yielding a five-banded alkaline spectrum in the urine of rheumatic patients, and added that he had also met with it in Pneumonia and Cirrhosis Hepatis. He stated however that the acid spectrum of this substance showed no band between *b* and *F*, provided that the solutions were free from impurities.

In 1889 MacMunn[5] described more fully than before the spectroscopic and chemical properties of urohæmatoporphyrin, and discussed its relationship to other urinary pigments. In reply to Le Nobel he maintained that the band before *F* is an essential part of the acid spectrum, and brought forward various arguments in support of his

[1] *Wiener Sitzungsberichte*, Abth. III. LXXXIV. 1881, p. 536.

[2] *Proc. Royal Soc.*, 1883, XXXV. p. 394. *Brit. Med. Journal*, 1883, ii. p. 1060.

[3] *Journal of Physiology*, 1885, VI. p. 636.

[4] *Pflüger's Archiv*, 1887, XL. p. 501.

[5] *Journal of Physiology*, 1889, X. p. 71; see also *Brit. Med. Journal*, 1888, I. p. 283; 1888, II. p. 117 and 1891, I. p. 3.

contention. He also added several fresh diseases to the list of those in which the pigment is met with in the urine.

In the same year (1889) Stockvis[1] described a colouring matter found by him in the urine of an elderly woman, who had taken sulphonal. The urine had a deep red colour, and the pigment contained in it yielded spectra practically the same as those of hæmatoporphyrin. Nevertheless, on account of its behaviour to certain solvents of that substance, Stockvis concluded that it could not be identical with it.

In 1890, Ranking and Pardington[2] added two fresh observations to the series. Both cases occurred in the same house, and both patients were females who exhibited obscure nervous symptoms, but are not stated to have taken sulphonal.

The urines, which had a deep red colour, were examined by Russell, MacMunn and Copeman[3] who agreed in pronouncing the colouring-matter to be hæmatoporphyrin, or a closely allied pigment.

In the same year Vaughan Harley[4] described two somewhat similar cases, but the spectroscopic appearances in the second case, which alone are recorded, are puzzling if, as seems probable, these were examples of hæmatoporphyrinuria. Both patients had taken sulphonal.

In 1891, Salkowski[5] described in detail the chemical and spectroscopic examinations of the dark red urines of three patients, all of whom had taken sulphonal. Salkowski discussed the methods which had been found most satisfactory for the separation of the pigment present in the urines, which he regarded as identical with the hæmatoporphyrin of Nencki and Sieber, having conducted a series of control experiments with normal urine to which that variety of the pigment had been added. He was inclined to attribute the presence of the pigment to the administration of sulphonal. A few months later Hammersten[6] published the results of the examination of the urine of four more patients, all of whom had been treated with sulphonal. His efforts were mainly directed to the isolation of the hæmatoporphyrin from the urine in a state of purity; and they were rewarded by success in obtaining it in a crystalline form in two cases; the crystals obtained

[1] *Nederland. Tijdschrift voor Geneeskunde*, 1889, II. p. 413.

[2] *Lancet*, 1890, II. p. 607.

[3] *Trans. Path. Soc.* XLII. 1891, p. 364.

[4] *Brit. Med. Journal*, 1890, II. p. 1169.

[5] *Zeitschrift f. Physiol. Chemie*, 1891, XV. p. 286.

[6] *Upsala Läkareförenings Förhandlingar*, 1891, XXV. and XXVI. *Skandinavische Archiv f. Physiol.*, 1891, III. p. 319.

resembling those of the compound of hæmatoporphyrin with hydrogen-chloride prepared by Nencki and Sieber[1].

Yet other cases were reported by MacMunn and by Halliburton in the *Proceedings of the Physiological Society* for 1890 and 1891, and very recently by Adolf Jolles[2] and Sobernheim[3].

The above brief summary does not pretend to do more than indicate where the literature of the subject of this paper is to be found, for it would be useless to attempt to do justice in a limited space to the many valuable and interesting observations contained in the papers above referred to. Of some of these, however, I shall have occasion to speak later.

My own observations and experiments which I will now proceed to describe, embrace examinations of the urine of upwards of a hundred and fifty patients and healthy individuals, for hæmatoporphyrin or allied pigments; a series of control experiments with urine to which hæmatoporphyrin had been added; a study of the changes produced in the spectra of hæmatoporphyrin by dilution, by different solvents, and by differences of acidity and alkalinity; a comparison of different methods of obtaining the pigment from the urine, including a new method not hitherto described; a study of the spectroscopic, and as far as possible, of the chemical properties of the specimens obtained, and lastly, attempts to obtain urinary hæmatoporphyrin in crystalline form.

The observations show that hæmatoporphyrin in some of its varieties is an extremely common urinary pigment; that it is usually present in extremely minute quantities, in the urine of healthy individuals, and that in a large number of morbid conditions considerable amounts are present, without materially affecting the colour of the urine.

Methods of detecting Hæmatoporphyrin in the Urine.

I have had no opportunity of examining any of the remarkable dark red urines which have formed the subject of several of the researches which have been referred to above, and my observations have been entirely confined to specimens exhibiting no striking peculiarity of colour. In some instances I have inferred correctly from the tint that hæmatoporphyrin was present in considerable amount, but when only

[1] *Archiv f. Experimentelle Pathologie u. Pharmakol.*, 1888, xxiv. p. 430.

[2] *Internat. Klinische Rundschau*, 1891, v. pp. 1914 and 1954.

[3] *Deutsche Med. Wochenschrift*, 1892, p. 566.

traces are present its detection by such means is quite impossible. I have also frequently noticed a pink tinge in the fluid vein produced by pouring an urine fairly rich in hæmatoporphyrin from one vessel to another. Such urine may also be rendered distinctly pink by the addition of a few drops of a mineral acid, and may show when examined with the spectroscope a pair of bands closely resembling those yielded by a dilute solution of oxyhæmoglobin, such as have been described by several observers, although the presence of unchanged blood pigment may be absolutely excluded. Hammarsten is disposed to assign these bands to some præcursor of hæmatoporphyrin; but a similar spectrum is seen when a very dilute neutral solution of the pigment is examined, the two bands in question being the darkest of the five which constitute the complete neutral spectrum, and I am inclined to agree with Stockvis that these are most probably the bands seen in the urine; for it is a well-established fact that, even when the urine is acid, the spectrum of acid hæmatoporphyrin is only brought out by the addition of a mineral acid.

In order to obtain the pigment in more concentrated solution, and, in the great majority of instances, in order to detect its presence, it is necessary to precipitate it and to prepare an acidulated alcoholic extract from the precipitate.

The precipitation may be effected in various ways. MacMunn recommends that the urine be decolorized with the neutral and tribasic acetates of lead, but this method has the disadvantage of yielding a very impure extract containing in solution all the pigments of the urine. Salkowski recommends the employment of the chloride of calcium or of barium, both of which metals form insoluble compounds with hæmatoporphyrin.

In all my earlier work I employed the lead, or the barium process, and when the latter method was employed equal parts of a saturated solution of barium hydrate, and of a 10 % solution of barium chloride were added to the urine, according to the directions given by Salkowski. In making use of this method care must be taken that the barium salt is present in excess, but even when this is the case traces of hæmatoporphyrin may often be detected in the filtrate from the barium precipitate, by means of the acetates of lead.

The great advantage of the barium method is that it does not by any means decolorize the urine, the bulk of the pigments other than hæmatoporphyrin passing on into the filtrate. If little urobilin be present the extract obtained may be quite free from that substance

provided that the precipitate has been washed with water and alcohol. If, however, there be much urobilin, and especially pathological urobilin, present, a considerable amount may be retained by the rather bulky precipitate, and in this way the purity of the alcoholic extract is seriously impaired.

Latterly, I have employed a method which yields considerably purer extracts than either of those above referred to, and which is so delicate as to allow of the detection of traces of hæmatoporphyrin in urines which, when examined in other ways, appear to be quite free from that pigment.

The method is as follows:—A small quantity of a solution of sodium or potassium hydrate (a 10 % solution answers very well) sufficient to render the liquid strongly alkaline, but not sufficient to destroy a filter-paper, is added to the specimen of urine. If the urine contains hæmatoporphyrin all or nearly all of the pigment will be carried down by the precipitate of earthy phosphates which forms. If the phosphate precipitate is scanty it should be increased in amount by the addition of a small quantity of a solution of calcium phosphate in water acidulated with hydrochloric acid.

The precipitate is then removed by filtration, washed off the filter into water, and again filtered off, this process being repeated until the washings are colourless.

If much uroerythrin be present the precipitate will have a dirty green colour, but thorough washing removes this pigment to a great extent, without apparently interfering with the hæmatoporphyrin.

If a considerable quantity of hæmatoporphyrin be present, the translucent precipitate, when washed, will have a delicate pink colour, and if examined through the filter, against a strong light, will show the alkaline spectrum of that substance.

After the water has drained off completely, the precipitate is treated upon the filter, with alcohol acidulated with sulphuric acid. The extract so obtained may exhibit the peculiar pink colour of hæmatoporphyrin, or may be yellowish pink or even yellow, according to the proportion between the amount of hæmatoporphyrin contained in it, and that of the impurities which are always present in small quantities, even after very thorough washing.

These impurities may, as a rule, be got rid of by shaking the extract with chloroform, after the addition of water, when the hæmatoporphyrin is usually taken up by the chloroform either from acid, neutral or alkaline solution, whilst the supernatant liquid retains a

yellow tint, but shows no absorption bands. Indeed the original extracts are spectroscopically pure, or at least show no urobilin band, provided that the precipitate has been washed.

When the patient has taken rhubarb or senna, and the urine consequently contains chrysophanic acid, the pink colouring matter which appears on the addition of soda adheres to the phosphate precipitate, imparting to it a bright bluish pink colour, not removed by washing, which might be mistaken for that of hæmatoporphyrin; but the tint is not identical, and such a precipitate does not show the characteristic spectrum. Any doubt is at once removed on the addition of the acidulated alcohol, which converts the pink colour to a bright yellow. Such an extract will show the hæmatoporphyrin bands when that pigment is present, but on adding ammonia the pink colour returns, and a broad area of absorption is observed in the centre of the spectrum. Chloroform takes up the chrysophanic acid from the acid extract in preference to the hæmatoporphyrin, and in this way a more or less complete separation of the two may usually be effected.

If the urine contains blood pigment this will be carried down by the phosphate precipitate (Heller's test), and the extract will exhibit the spectrum of acid hæmatin.

Unfortunately there is an element of uncertainty in the process, and whereas extracts obtained by precipitating the filtrates with barium chloride, have usually been quite free from hæmatoporphyrin, and at other times have shown traces only, it has occasionally happened that the bulk of the pigment, or even the whole of it has been found to have escaped precipitation by the soda. I hope that further experience of the process will enable me to find out upon what this very exceptional conduct of the pigment depends, and to obtain a safeguard against such accidents. In some instances the failure could be distinctly traced to the addition of an insufficient quantity of the alkaline solution.

The small bulk of the precipitate allows of the complete removal of the pigment from it by a very small quantity of acidulated alcohol, and the extracts are therefore richer in hæmatoporphyrin than those obtained by other methods. As a result the proportion of cases in which a complete alkaline spectrum can be obtained is very much larger than when other methods are employed.

The precipitate of phosphate plays an essential part in the process, for the sodium and potassium compounds of hæmatoporphyrin are soluble in water. When a specimen of hæmatoporphyrin prepared by

the sulphuric acid process, is largely diluted with water, and rendered alkaline with sodium or potassium hydrate the solution remains clear, and passes through a filter without leaving anything upon it. When however a small quantity of an acid solution of calcium phosphate is added, the pigment adheres to the precipitate, and is not removed from it by washing, whilst the filtrate no longer shows the hæmatoporphyrin spectrum.

The substitution of ammonia for soda or potash gave very unsatisfactory results, only a trace of hæmatoporphyrin being obtained from a urine rich in that substance; whilst an urine in which the soda process revealed a trace gave a negative result with ammonia.

For the detection of hæmatoporphyrin it is not necessary to examine large amounts of urine. The quantities employed in my examinations varied from 150 c.c. to 350 c.c., but for the purposes of more complete study of the pigments present, much larger amounts, up to a litre, were taken.

The acidulated alcohol extracts varied in bulk from 5 to 20 c.c., and were examined spectroscopically in depths of from 25 to 45 millimeters.

General characters of Hæmatoporphyrin obtained from urine.

The pigments obtained from urine, *when pure*, yielded acid solutions exhibiting the peculiar pink colour which is characteristic of hæmatoporphyrin, and when such solutions were compared with others prepared from blood no difference could be detected between them.

The neutral solutions were paler but had a very similar tint, and the alkaline solutions were also pink, like those of the alkaline hæmatoporphyrin of Nencki and Sieber, and had not the brown-pink colour usually observed in alkaline solutions of the pigment prepared by means of sulphuric acid. It is indeed remarkable that the acid, neutral and alkaline solutions resemble each other so closely in tint, in spite of the great differences observed in the distribution of their absorption bands.

I am inclined to think that the brown-pink colour of Hoppe-Seyler's hæmatoporphyrin in alkaline solution may be due to impurities, for my sulphuric acid specimens have occasionally exhibited when in alkaline solution the same beautiful pink colour as is seen in alkaline solutions of the urinary pigments.

As in Salkowski's cases, the behaviour of the urinary pigment to chloroform was somewhat capricious, and appeared to be determined by

a variety of causes, such as the purity of the solution, or the amount of acid or alcohol present. Sometimes it could only be got to go into chloroform out of neutral or nearly neutral solutions; in other instances it was only so removed from acid or alkaline solutions. With Hammarsten, I have frequently seen the pigment leave the chloroform entirely when the supernatant liquid was rendered alkaline.

A pigment which on many occasions refused entirely to go into chloroform, has on another been readily taken up; and a pigment which was at first untouched by chloroform, has been readily taken up after a second precipitation of the extract. It would therefore seem that the behaviour of the extracts when shaken with chloroform affords no true indication of the solubility of the pigment in that liquid; and that it would be erroneous to conclude from any peculiarities of behaviour under such circumstances that the various specimens are chemically distinct from each other.

When the chloroform solutions were allowed to evaporate the pigment was left as a brown amorphous residue. In my cases this residue was always completely soluble in cold absolute alcohol, and I have not as yet met with any specimen, in which, as in two of Hammarsten's cases, a portion was insoluble in cold, but soluble in hot alcohol. It is probably for this reason that I have hitherto failed to obtain the pigment in a crystalline form either from its solutions in chloroform or in ethylic or amylic alcohol. The alcohol solutions have always deposited the pigment in amorphous form, usually in tiny spherules.

These evaporation experiments are apt to produce a change in the pigment, which may no longer show the absorption bands of hæmatoporphyrin, exhibiting instead a fresh set of spectra in acid alkaline and neutral solution, although to the naked eye the colour of the solutions is not perceptibly altered. Similar changes were observed in specimens prepared from blood, which were treated in the same manner.

It is a noteworthy fact that the processes which serve for the precipitation of the pigments from urine, are equally efficacious when applied to urine to which hæmatoporphyrin has been added; and in this we have further evidence that if they be not chemically identical with the product obtained from blood they are at least very closely related to it. Salkowski has shown that this is true of urine containing the hæmatoporphyrin of Nencki and Sieber, and I have frequently verified it with specimens containing the pigment obtained by the action of sulphuric acid upon hæmoglobin.

The extremely small quantities of hæmatoporphyrin present even in those of my specimens which were most rich in the pigment rendered any complete study of the chemical properties of the urinary products extremely difficult; and, coupled with my want of success in obtaining crystals, rendered any attempt to obtain an ultimate analysis out of the question.

Spectroscopic characters of the urinary Pigments.

In their spectroscopic characters the pigments obtained from different cases presented slight differences in the positions of the absorption bands, and as similar differences were observed in specimens of hæmatoporphyrin prepared from blood pigment it seemed desirable to ascertain, as far as possible, whether they were inherent in the pigments themselves or were attributable to external causes.

The first point studied was the effect of dilution upon the spectra, since the changes produced by this cause are important when solutions of varying degrees of concentration are being examined.

In concentrated solutions of acid hæmatoporphyrin the two distinctive bands are separated by a narrow interval near the *D* line, and some shading is seen between *E* and *b*, which falls off rapidly towards the blue end of the spectrum. This shading is soon removed by dilution. (Fig. 1.)

In aqueous solutions the first band reaches to and just encloses the *D* line, and the second band is preceded by a broad shading which at this stage appears to be of equal depth throughout. As dilution proceeds the darker portion of this shading, which is near its less refrangible border, becomes detached from the second band, and appears as an independent faint band. (Fig. 2.)

Meanwhile both the dark bands shrink from their less refrangible ends, the borders towards the violet being scarcely altered at all. After a certain degree of dilution is reached no further shrinkage takes place; the intermediate shading gradually fades and disappears, as ultimately does the first band also, a mere shadow of the broader second band alone remaining visible.

It will be obvious from the above description that the more refrangible borders of the bands are the more important for purposes of identification, and that dilution produces conspicuous changes both in the breadth of the bands and in the positions of their centres.

In the alkaline spectrum the phenomenon of shrinkage is less marked; the shading before the second band fades away as dilution

proceeds, but the breadth of the bands is little altered as long as they remain visible.

The band in red is the first to disappear. As with the acid spectrum the more refrangible borders of all the bands are less affected by dilution than those towards the red.

The nature of the solvent has a very important influence upon the positions of the bands. It was noticed that when acid hæmatoporphyrin, either artificial or urinary, was dissolved in chloroform a remarkable displacement of all the bands towards the red was sometimes but not always observed.

That this shifting was not dependent upon acidity was proved by the fact that when the supernatant liquid was rendered alkaline and again shaken with the chloroform the bands of the alkaline spectrum showed a similar displacement towards the red.

I soon found that the inconsistency of the results was due to the admixture of alcohol with the chloroform, and that when much alcohol was present the shifting of the bands was very slight or inappreciable, but that when the alcohol was washed out with distilled water it at once became conspicuous. By successive additions and removals of the alcohol it is possible to shift the bands backwards and forwards within certain limits at will.

The amount of displacement bears some relation to the molecular weight of the solvent, but was not found to be proportional thereto. Nor are the several bands shifted to an equal extent, for in going from alcohol to chloroform the second band of the acid spectrum is displaced more than the first; whereas on going into bromoform the two are shifted to an equal extent.

The following table shows the displacement of the bands of acid hæmatoporphyrin by various solvents; only the positions of the more refrangible borders of the bands being indicated. Errors due to varying degrees of acidity were excluded by repeating the observations with fresh specimens.

Solvent	Molecular weight	Band α	Band β
Water	18	λ 5875	λ 540
Ethyl alcohol	46	λ 5905	λ 543
Amyl alcohol	88	λ 5905	λ 543
Chloroform	119·5	λ 592	λ 549
Bromoform	253	λ 599	λ 552

It will be seen that the positions of the bands were the same in ethyl and amyl alcohol although the molecular weight of the latter is nearly twice as great as that of the former.

When two specimens of an aqueous solution of acid hæmatoporphyrin (Fig. 1) are brought into direct comparison before the spectroscope and alcohol is added to one of them the bands are shifted towards the red end of the spectrum. When two alkaline aqueous solutions (Fig. 4) are similarly compared, and alcohol is gradually added to one of them, the first effect of the addition is to shift all four bands towards the blue; but when a larger amount of alcohol is added the first two bands return into agreement with those of the aqueous solution although the third and fourth bands remain displaced towards the blue. When a pure rectified spirit solution is compared with an aqueous one, the above described shifting of the third and fourth bands towards blue is observed, but the first band is also slightly displaced towards the red, with the result that the alcoholic spectrum is more spread out in both directions than the aqueous.

The degree of acidity or alkalinity of the solutions has also an important influence upon the spectra. Addition of acid shifts the bands of the acid spectrum towards the red: whereas when the amount of alkali is increased the bands of the alkaline spectrum are displaced towards the violet.

The importance of the above phenomena is at once apparent when it is considered that unless the precipitates have been completely dried before extraction, the extracts examined consist of mixtures of alcohol and water in varying proportions, with varying proportions of acid and alkali; and it follows from this that slight displacements of the absorption bands are due to such accidental causes rather than to any abnormality in the pigments themselves.

The Acid Spectrum.

The spectra of the urinary pigments when in acid solution were exactly similar to that of hæmatoporphyrin prepared from blood[1]. The

[1] All my specimens of hæmatoporphyriu were prepared by acting with strong sulphuric acid upon Pfeufer's Hæmoglobin (the drug), which affords a very convenient source of blood pigment. (In aqueous solution it gives the spectrum of oxyhæmoglobin.) In order to purify the product it may be filtered through asbestos, and precipitated by neutralization in an excess of water. The precipitate formed may be filtered off, washed upon the filter and redissolved in acidulated alcohol; or it may be precipitated from a solution rendered alkaline with potassium and sodium hydrate, by the addition of a small quantity of an acid solution of calcium phosphate.

acidulated extracts usually showed the two characteristic dark bands, one just before the *D* line and the other between *D* and *E* (I. λ 597—587. II. λ 557—λ 541), and an intermediate faint band, which represents the darkest portion of the shading, which with more concentrated solutions leads up to the second band. (This shaded band usually reads λ 576—λ 570 or λ 567.) When the colour of the solution is more intense the urinary, like the artificial pigment, shows a continuous shading up to the second band, and in very concentrated solution it also shows the faint shading between *E* and *b*, which is shown by strong solutions of hæmatoporphyrin from blood. (Pl. XXIV. Fig. 1.)

When the solutions approached to a condition of purity the above described bands constituted the entire acid spectrum of the pigments obtained from my cases. The extracts from the barium precipitate usually showed some shading in the position of the urobilin band, even after careful washing, but in some few of these the blue end of the spectrum was clear.

Extracts from the soda precipitates, provided that the precipitates had been washed, never showed any band or shading in the position of the urobilin band, such as MacMunn describes as forming an essential part of the spectrum of acid urohæmatoporphyrin.

The Neutral Spectrum.

When chloroform solutions of the urinary pigments were washed with distilled water, and cleared by the addition of a little alcohol, or when the chloroform was allowed to evaporate and the residue was dissolved in alcohol, spectra were obtained identical with those of neutral hæmatoporphyrin prepared by the same methods.

The absorption bands were five in number; two between *C* and *D*; two between *D* and *E*; and one between *b* and *F*. Of these, as has been already mentioned, the third and fourth were the most conspicuous, whilst the first and fifth are comparatively faint (Fig. 3).

When the solution was concentrated the broad third band had a fluted appearance, being composed of a series of three darker and three fainter portions.

The Alkaline Spectrum.

The various specimens of urinary hæmatoporphyrin yielded, when in alkaline solution, spectra conforming to two different types.

In the great majority of my cases the alkaline spectrum exhibited

the four absorption bands which are described as characteristic of hæmatoporphyrin in alkaline solution (Fig. 4), but in a certain number of instances a five-banded spectrum was observed identical with that assigned by MacMunn to alkaline urohæmatoporphyrin (Fig. 5) but differing essentially from the five banded neutral spectrum (Fig. 3).

Both kinds of spectra were met with in the extracts from the urines of different patients suffering from the same disease, but repeated examinations of the urine of the same individual always gave similar results.

The fifth band, when present, was situated near the *C* line of the solar spectrum, and was slightly broader than the second band, which corresponded with the band in red of the neutral spectrum.

It is often a matter of much difficulty to be sure whether or no the extra band is present, especially if the entire spectrum is faint. This difficulty arises from the fact that the shading at the red end of the ordinary four-banded spectrum, both of the urinary and blood derivatives, ceases abruptly at the exact line where the more refrangible border of the extra band should be, and so may suggest the presence of such a band.

I am now convinced that in some of my earlier cases I was deceived by this appearance, and so was led to form too high an estimate of the frequency of the five-banded spectrum; but in a few instances the specimens showed the fifth band so clearly that there could be no doubt as to its presence. It is always difficult to measure accurately the more refrangible border of this band, because it is more or less merged in the shading at the red end of the spectrum. In the tables which follow I have only described the spectrum as five-banded, in the cases in which the extra band was quite distinct.

The fifth band was sometimes much fainter than the others, and it seemed, in some cases, as if a small quantity of the five-banded pigment was mixed with a much larger quantity of the four-banded variety, the faint extra band alone affording evidence of its presence.

The five-banded spectrum above described is, as MacMunn has shown, identical with that of a variety of hæmatoporphyrin which is obtained by the action of nascent hydrogen upon hæmatin, and also by the reduction of ordinary hæmatoporphyrin. Le Nobel states that he also obtained it by shaking an alkaline solution showing four bands only, with acetone or aldehyde. I have tried to repeat this experiment with acetone both in the cold and on the water bath, but hitherto without success.

The pigment obtained by the action of nascent hydrogen upon hæmatin or hæmatoporphyrin shows, when in acid solution, a dark band before *F* like the urobilin band, and MacMunn adduces this fact as additional evidences of the presence of a similar band in the spectrum of acid urohæmatoporphyrin. As the process of reduction proceeds the acid hæmatoporphryin bands fade whilst the band before *F* becomes darker and darker, and ultimately is alone visible, the pigment named urobilinoidin by Le Nobel, having been produced.

When preparing hæmatoporphyrin by the sulphuric acid method I have, in the great majority of instances, obtained a product which, when rendered alkaline, exhibited the ordinary four-banded spectrum, but on one or two occasions the alkaline spectrum has been five-banded, and exactly like that which is sometimes seen with the urinary specimens. A specimen of this kind when reacidified with hydrochloric acid showed the acid hæmatoporphyrin bands and an ill-defined shading in the blue, which was too faint for measurement, but extended approximately from λ 517—477. This product was very unstable, and when taken up by chloroform and amyl alcohol gave the four-banded alkaline spectrum.

In one of his papers MacMunn states that hæmatoporphyrin in alkaline solution may exhibit four or five bands[1], and it is important to note that he also describes urohæmatoporphyrin as sometimes yielding a four-banded alkaline spectrum, and ascribes such variations to very trifling differences[2].

The occurrence of the two types of pigments in the urine of sufferers from the same disease lends strong support to this view, and it seems probable that in some cases the pigment has undergone a greater or less amount of reduction before being excreted.

Of all the absorption bands of hæmatoporphyrin the less refrangible bands of the alkaline spectrum were those which most frequently exhibited slight displacements, and it appears to me that this fact receives a sufficient explanation from the observations above described of the effects of the admixture of alcohol and water in the solutions.

In the spectra of some of my specimens, both urinary and artificial, there was yet another band seen in the extreme blue, reading approximately λ 469—λ 455. This band was only observed when an excess of ammonia was present.

[1] *Brit. Med. Journal*, 1891, i. p. 6.

[2] *Journal of Physiology*, x. 1889, p. 79.

The Spectrum with Zinc Chloride and Ammonia.

When zinc chloride and ammonia are added to a solution of hæmatoporphyrin, either artificial or ordinary, a spectrum is seen consisting of two bands between *D* and *E* (Fig. 6). If only small amounts of the reagents are employed the ordinary alkaline spectrum is at first seen, and the characteristic bands are only developed when the solution has been allowed to stand for some hours. The band in red quickly fades away, but that in blue disappears much more slowly. The two characteristic bands read as follows: λ 586—λ 570 and λ 552—λ 532. When the solution is a concentrated one a faint shaded band is seen lying midway between them.

The above observations which were independently made are in complete accord with those of Hammarsten.

MacMunn describes the spectrum of urohæmatoporphyrin treated with zinc chloride and ammonia as consisting of two bands identical in position with those above described, and a third which agrees with the band observed when a solution of normal urobilin is similarly treated. With hæmatoporphyrin prepared from blood he obtained only the ordinary alkaline spectrum on the addition of these reagents.

When the solutions so treated were pure I found that their tint was indistinguishable from that of the ordinary alkaline solutions, in spite of the great differences in the distribution of the absorption bands. No fluorescence was observed in any specimen which could be shown to be free from urobilin.

In illustration of the points above referred to I may give brief descriptions of the results obtained in two particular cases.

Case I. J. B. a painter, aged 30 years, was suffering from his second attack of gout which had commenced three days previously. He had a tophus upon the pinna of one ear, and a blue line upon the gums.

The urine of this patient was examined upon ten different occasions, but as the results of all the examinations agreed I will give only a summary of them. The urine was once precipitated with lead acetate, seven times with barium chloride, and twice with soda. There was a considerable amount of hæmatoporphyrin present on all occasions.

The urine, as passed, showed two faint bands resembling those of oxyhæmoglobin, but the presence of that pigment could be excluded. It was pale in colour, and slightly turbid.

The acid spectrum of the isolated pigment read:

λ 597—587.

Shading λ 576—567.

λ 557—541.

No band between *b* and *F* was ever observed except on the first occasion when the lead method was employed.

On some occasions the pigment went into chloroform yielding a solution of the peculiar hæmatoporphyrin-pink colour. The chloroform solution when washed showed a five-banded neutral spectrum reading:

λ 622—613.

λ 608—597.

λ 573—552.

λ 540—519.

λ 513—484.

With ammonia the solution remained pink, and the spectrum was always four-banded, reading as follows:—

Shading ceasing abruptly at λ 638.

λ 622—614.

λ 597—579—563.

λ 541—526.

λ 513—491.

With zinc chloride and ammonia two bands were seen reading:—

λ 586—570.

λ 552—532.

The solutions exhibited no fluorescence.

The chloroform solution of the pigment from a solution acidulated with hydrochloric acid was washed with water, and allowed to evaporate to dryness upon a watch-glass; a brown amorphous deposit being left. The residue was completely dissolved by cold alcohol, which on evaporation again deposited the pigment in an amorphous form.

The pigment present in this case was liable to undergo changes. Thus a specimen of the sodium extract which when rendered alkaline with ammonia yielded the ordinary four banded-spectrum, after being kept for five days, showed only a group of three entirely new bands reading as follows:—

λ 582—573.

λ 563—555.

λ 549—535.

The change which here took place must have been a very slight one, for on adding hydrochloric acid to the specimen the acid hæmato-

porphyrin spectrum appeared, and the addition of ammonia then brought out the original four-banded alkaline spectrum.

Case II. John S. aged 38 years, suffering from general œdema for which no satisfactory explanation could be discovered, was admitted to the West London Hospital under the care of my colleague Dr Donald Hood, to whom I am indebted for the opportunity of making the examinations. His urine was copious, of a pale yellow colour, and contained no albumen. It was examined by the soda method on three occasions, in quantities varying from 100 to 700 c.c.

The soda precipitate, when washed, had a bluish pink colour, and when examined through the filter with a small direct vision spectroscope showed the alkaline hæmatoporphyrin spectrum very distinctly.

The acidulated alcoholic extract had a beautiful pink colour, and showed the acid hæmatoporphyrin bands with great intensity, and the shading between *E* and *b*, but there was no band or shading whatever before the *F* line.

λ 599—587.
Shading 576—557.
557—541.

In concentrated solutions the shading was continuous with the second band, but was more intense towards its less refrangible border. (Fig. 1.)

On the addition of ammonia the solutions remained pink, but the four bands of alkaline hæmatoporphyrin were very conspicuous. There was moreover an extra band in the red, considerably fainter than any of the others, but nevertheless quite distinct. (Fig. 5.)

λ 652—638 faint.
624—613.
597—582—560.
540—524.
511—489.

With zinc chloride and ammonia two bands were seen after the solution had stood for a few hours, during which time a third band in blue which was at first visible disappeared. A faint shaded band was also observed midway between the two characteristic bands. (Fig. 6.) The bands read:

λ 586—570.
549—532.

The solution retained its pink tint.

A concentrated neutral alcoholic solution of the pigment, which,

like the above-mentioned solutions, was pink in colour, showed a five-banded spectrum (Fig. 3), and the fluted character of the third band was very conspicuous. The readings were:—

λ 624—613.
604—599.
582—573—552.
537—520.
511—484.

The pigment was readily taken up by chloroform, and when the chloroform solution was allowed to evaporate upon a watch glass, was deposited in minute spherules which ran together forming amorphous masses. It was entirely dissolved by cold absolute alcohol which on evaporation again deposited it in an amorphous form.

Amyl alcohol also dissolved it completely and on evaporation deposited it in an amorphous state.

The specimen which had been submitted to the above processes was dissolved in absolute alcohol, and examined with the spectroscope. Although it still retained its pink colour apparently unaltered, its acid, alkaline and neutral solutions no longer yielded the hæmatoporphyrin spectra, but showed a fresh set of bands, reading as follows:—

Acid.	λ 592—589.	Alkaline.	very faint	λ 647—633.
				622—612.
	570—555.		very dark	569—555.
	faint 532—517.		faint	537—517.
				503—487.

The altered pigment was readily taken up by chloroform.

I have observed a series of spectra practically identical with these after submitting a specimen of hæmatoporphyrin prepared from blood to the same treatment.

Since, as will be seen from the descriptions on p. 617, the pigments met with in urine exhibit such very close resemblances to hæmatoporphyrin, and do not differ from specimens prepared from blood pigment any more widely than such specimens differ among themselves, it seems to me that we cannot refuse to them the name of hæmatoporphyrin.

I have employed this name when speaking of the pigment rather than that of urohæmatoporphyrin because, although my observations agree with those of MacMunn as regards the occasional presence of a fifth band in the alkaline spectrum, I am compelled to differ from him as to the presence of a band before *F* in the acid spectrum, which he regards as an essential feature of the spectrum of acid urohæmatopor-

Hæmatoporphyrin in Urine. Specimen Readings, in Wave Lengths.

Sex and Age		G. B. Male. 30	G. B. Male. 64	G. S. Male. 58	J. S. Male. 38	W. B. Male. 7	L. G. Female. 15
Disease	Urine with hæmatoporphyrin added	Gout	Cirrhosis Hepatis	Gout	General œdema. No albuminuria	Paroxysmal hæmoglobinuria, between paroxysms.	Chlorosis
Acid spectrum	λ 597—587 shading 576—570 558—541	λ 597—587 shading 576—556 556—541	λ 597—587 shading 576—570 557—541	λ 597—587 shading 576—567 556—541	λ 599—587 shading 576—557 557—541	λ 597—587 shading 576—570 557—541	λ 597—587 shading 576—567 557—541
Alkaline spectrum	shading to 640 λ 626—614 597—579—563 541—526 513—491	shading to 638 λ 622—614 601—573—558 541—526 511—491	shading to 638 λ 622—610 597—573—557 540—526 513—491	shading to 633 λ 620—610 597—573—560 541—526 511—493	λ 652—638 624—613 597—582—560 540—524 511—489	λ 652—638 626—616 597—576—561 542—527 513—491	shading to 640 λ 624—614 597—573—560 542—526 513—489
Neutral spectrum	λ 628—620 612—601 576—555 541—524 513—486	λ 622—614 608—597 573—552 540—519 513—484	λ 628—618 606—594 576—552 540—519 511—484	λ 624—614 606—601 579—552 540—526 513—489	λ 624—613 604—599 582—573—552 537—520 511—484	λ 626—618 606—599 576—552 540—524 513—484	λ 626—616 608—597 576—555 541—526 511—489
With Zinc chloride and Ammonia	λ 586—570 552—532	λ 586—570 552—532	λ 586—570 552—532	λ 586—570 552—535	λ 586—570 549—532	λ 586—570 552—532	λ 589—573 552—535

phyrin, but which I have found to be entirely absent when the specimens were pure or at least were free from any contamination with urobilin.

Although urobilin is taken up by chloroform with much more certainty than hæmatoporphyrin is, I have never succeeded in entirely separating the two pigments by this means; for even when, from a mixture of these pigments, the urobilin was taken up by chloroform whilst the hæmatoporphyrin was untouched, the supernatant liquid always retained a trace of the band before *F*, even when fresh chloroform removed no more urobilin. However the band was usually reduced to a faint shading.

The above description of urinary hæmatoporphyrin is based upon the examination of some 160 urines of healthy individuals and sufferers from various diseases.

Among 130 urines examined by the lead and barium methods there were eighty in which the extracts showed the bands of hæmatoporphyrin in acid solution, but only fourteen in which the alkaline spectrum could be obtained; whereas of thirty consecutive urines examined by the soda method, twenty-nine showed the acid spectrum more or less distinctly, and in no less than nineteen the complete alkaline spectrum was observed. Among the diseases in which the pigment was most frequently present in considerable amounts were Gout, Rheumatism, Chorea, Tubercular affections, Lobar Pneumonia and Pleurisy.

The frequency with which the bands of the acid spectrum were seen although the amount of hæmatoporphyrin present was not sufficient to give the alkaline spectrum, renders it necessary to consider whether the acid spectrum alone may be accepted as affording sufficient evidence of the presence of hæmatoporphyrin. I am myself convinced that it may, for the following reasons:—

The pigments which yield the faint acid spectra are precipitated by soda and potash as hæmatoporphyrin is, and are not removed from the phosphate precipitate by washing.

Traces detected in small specimens of urine were sometimes shown to be undoubtedly hæmatoporphyrin by the examination of larger quantities.

The more concentrated and purer soda or potash extracts yielded the complete alkaline spectrum much more frequently than did the extracts from the lead or barium precipitates.

Lastly, in many cases in which the complete alkaline spectrum could not be obtained the darker bands of that spectrum were seen, or the characteristic bands were observed on the addition of zinc chloride.

Hæmatoporphyrin in the urine of healthy individuals.

Usually, but not always, the urine of healthy persons was found to contain traces of hæmatoporphyrin when examined by the soda or potash method, although it was very seldom detected in such urines by the lead or barium method.

The quantity of urine taken varied from 200 to 400 c.c. and a small amount of extract (about 5 c.c. was prepared). With the spectroscope very faint shadows of the acid bands were usually seen, too faint for accurate measurement, but obviously occupying the correct positions. Sometimes traces detected by a small direct vision spectroscope, were invisible when an instrument of greater dispersive power was employed.

In order to put the value of such evidence of the presence of hæmatoporphyrin to a conclusive test the following experiment was performed.

The urine of a healthy man had been repeatedly examined by the lead and barium methods, always with negative results, but had always shown minute traces when the soda method was employed.

When two specimens passed on the same day were examined by the soda and barium processes the soda extract showed very faint bands, the barium extract none at all.

Of this urine about 600 c.c. were collected, including that secreted during sleep, and the specimen was precipitated with soda. By this means an extract was obtained which showed the acid spectrum distinctly. The solution was rendered alkaline and shaken with chloroform, which, contrary to expectation, took up the pigment readily, and showed a distinct alkaline hæmatoporphyrin spectrum.

There could no longer be any doubt that in this instance at any rate the pigment which yielded the extremely faint bands in the acid extracts from soda precipitates was actually hæmatoporphyrin, and the presumption was greatly strengthened that the same was true of other normal specimens in which similar appearances were observed.

I do not intend to enter here upon any discussion of the interesting question of the origin of urinary hæmatoporphyrin, because it appears to me that the evidence as yet forthcoming is quite insufficient for its solution. I hope that further study of the subject may supply firmer foundations for the construction of a theory to account for its presence in greater or less amounts, and will therefore reserve for the present such data bearing upon the question as my work has hitherto supplied. Especially does it seem probable that the power possessed by sulphonal

of causing in some cases the excretion of the pigment in large quantities, may serve to throw light upon the processes which lead to its appearance in the urine in various morbid conditions, and even in health. That sulphonal does actually possess this power it is hardly possible to doubt in face of the evidence which has recently been accumulated.

In conclusion it will be well to sum up briefly the results embodied in this paper.

1. A pigment is present in the urine both in health and disease which exhibits so close a resemblance to hæmatoporphyrin, that it must be at any rate regarded as one of the group of compounds included under that name.

2. The most delicate means of detecting this pigment in the urine is by precipitating with potassium or sodium hydrate (with the addition of calcium phosphate if necessary), and preparing an acidulated alcoholic extract from the precipitate. This process also yields a purer extract than any other hitherto proposed, but is unfortunately not absolutely certain in its action.

3. Acid solutions of urinary hæmatoporphyrin yield spectra identical with that of hæmatoporphyrin prepared from blood, and when free from urobilin show no band between *b* and *F*.

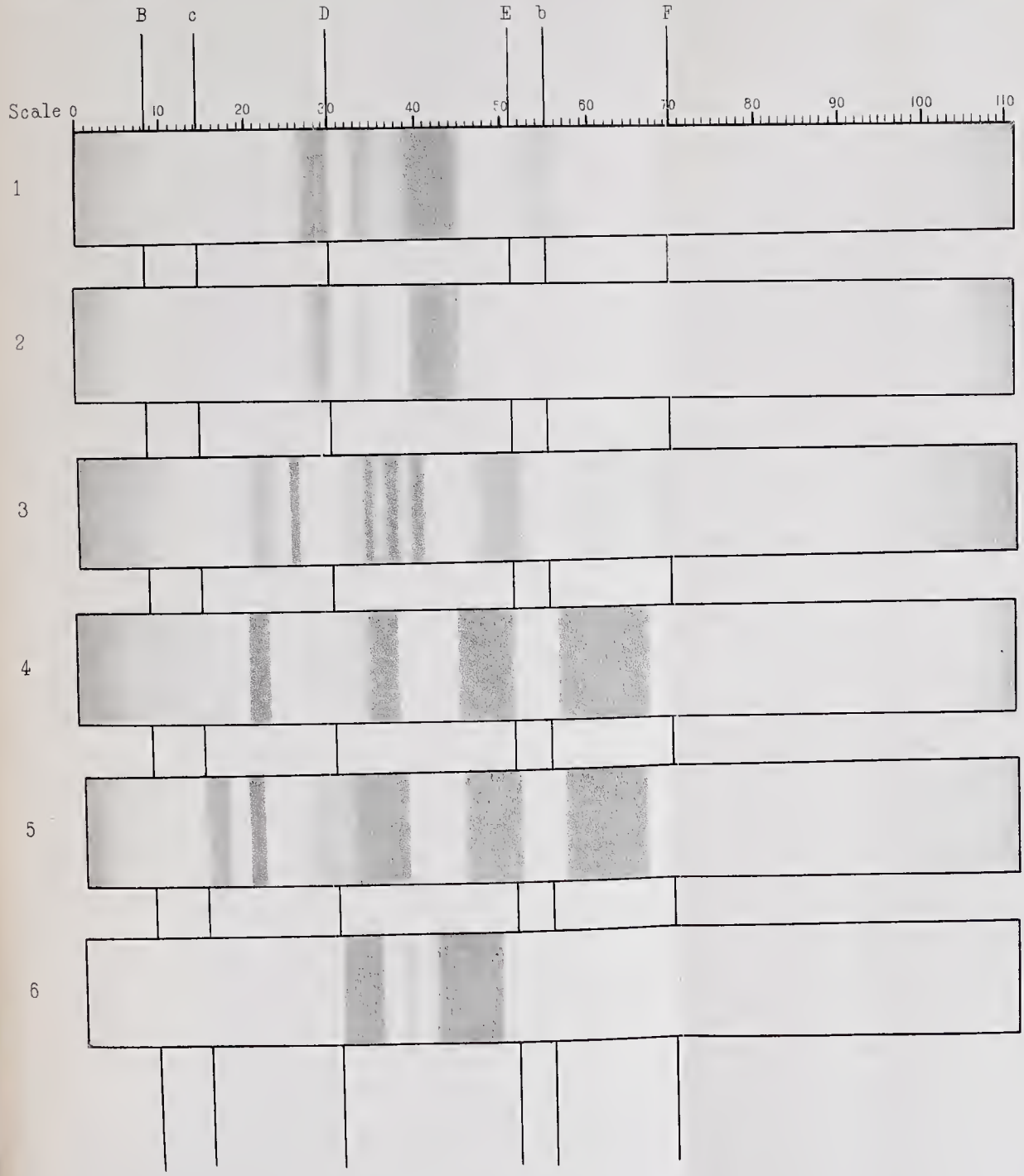
4. In alkaline solution the pigment usually exhibits the characteristic four-banded spectrum of hæmatoporphyrin, but the fifth band described by MacMunn is occasionally present, as in the spectrum of a product of the reduction of hæmatoporphyrin or of hæmatin, and of some specimens of hæmatoporphyrin prepared by the sulphuric acid process.

5. The neutral spectrum and the spectrum of solutions treated with zinc chloride and ammonia are identical with those yielded by hæmatoporphyrin prepared from blood, when treated in the same manner.

6. The urinary specimens, when pure, have the characteristic pink colour of hæmatoporphyrin both in acid, alkaline and neutral solution.

7. They exhibit differences of chemical behaviour comparable with those observed between specimens of hæmatoporphyrin prepared by different processes from blood pigment.

8. The pigment is frequently present in exceedingly minute quantities in the urine of healthy individuals, and in larger amounts in the urines of sufferers from a great variety of diseases.



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